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The rapid hydrolysis and efficient absorption of triglycerides with octanoic acid in the 1 and 3 positions and long-chain fatty acid in the 2 position[1,2]

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ABSTRACT We describe rapid hydrolysis of triglycerides with medium-chain fatty acids in the 1 and 3 positions and a long-chain fatty acid in the 2 position. The triglycerides, 2-linoleoyl-1,3-dioctanoyl glycerol (8L8) and 2-oleoyl-1,3-dioctanoyl glycerol, hydrolyzed more rapidly than triglycerides comprising all long-chain fatty acids. The in vitro hydrolysis rate of 8L8 was similar to that of a medium-chain triglyceride of octanoic and decanoic acids in random positions. From intestinal recovery of ^{14}C 45 min after injection into the isolated, irrigated loop of the small intestine of an anesthetized rat, the amount of 2-[1-^{14}C]linoleoyl-1,3-dioctanoyl glycerol absorbed was > 2½ times that of its long-chain analog, 2-[1-^{14}C]linoleoyl-1,3-dioleoyl glycerol. These data support the ease of hydrolysis and absorption of 1,3-dioctanoyl triglycerides with long-chain fatty acids in the 2 position. *Am J Clin Nutr* 1987;45:940–5.

KEY WORDS Medium-chain triglycerides, dioctanoyl triglycerides, essential fatty acids, pancreatic insufficiency, lipase

Introduction

The use of medium-chain triglycerides (MCT) of octanoic and decanoic fatty acids to provide a dense form of calories and the hedonic benefit of fat in patients with pancreatic insufficiency and other malabsorption problems is well documented (1, 2) and common practice. The ease of hydrolysis of MCT by pancreatic lipase and other esterases results in essentially complete absorption of these fats in cases in which long-chain triglycerides would be poorly absorbed and would produce steatorrhea. MCT fats do not, however, provide essential fatty acids (EFA), and the development of EFA deficiency in pancreatic insufficiency can occur during a dietary regimen that includes MCT (3).

We report here a preliminary investigation of the hydrolysis and absorption of 1,3-dioctanoyl triglycerides that can provide EFA in a form that provides the hydrolysis and absorption advantages of MCT. Pancreatic lipase specifically hydrolyzes the 1 and 3 positions of triglycerides to produce the free fatty acids from these positions and the 2-monoglyceride (4). If all fatty acids of the triglyceride are longer than decanoic acid, the fatty acid and monoglyceride products of hydrolysis form mixed micelles with bile salts and are absorbed by the mucosal membrane as fatty acids and 2-monoglyceride (4). The 2-monoglyceride is a well-absorbed form of most fatty acids since it readily forms mixed micelles with bile acids and since it cannot form insoluble soaps with divalent cations. We present data to show that the hydrolysis of octanoic acid from the 1 and 3 positions of a triglyceride and the subsequent absorption of the resulting monoglycerides are rapid in an animal model of pancreatic insufficiency.

Materials and methods

Materials

The 1,3-dioctanoyl and 1,3-diacetyl triglycerides were synthesized and isolated [silica gel column eluted with petroleum ether:ethyl ether:acetic acid, 90:10:1) by methods described previously (5). Triacetin (bp 132–154°C; Kodak,

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Rochester, NY), medium-chain triglyceride (67% octanoic acid, 23% decanoic acid, 10% other fatty acids; Mead Johnson, Evansville, IN), soybean oil (Crisco, Procter & Gamble, Cincinnati, OH), sunflower oil (Natural Sales, Pittsburgh, PA), and randomly mixed long- and medium-chain triglycerides (Captex 810 series; Capital City Products, Columbus, OH) were used. Tributanoin and tridecanoin were synthesized in our laboratories from the appropriate acyl chlorides as described previously (5) and isolated by column chromatography (5).

The Captex series of oils are triglycerides that are synthesized from mixtures of various ratios of long- and medium-chain fatty acids to form random structures. The fatty acid compositions of these oils are shown in **Table 1.**

The ^{14}C labeled materials were synthesized with [1-^{14}C]linoleoyl chloride (99% purity; New England Nuclear, Boston, MA) (5). Densitometric analysis of thin-layer chromatography (TLC) plates (eluted with hexane:ethyl ether:acetic acid, 140:50:2) showed these materials to be at least 99% triglyceride.

Radiochemical analysis of the TLC plates showed 98% of the ^{14}C to reside in the triglyceride region. The structures of these materials and of their ^{12}C analogs were confirmed by lipase digestion and isolation of the resulting 2-monoglyceride and fatty acid (6). Fatty acid distribution in the glyceride positions was determined by gas-chromatographic (GC) and radiochemical analyses of the fatty acid methyl esters (7) of the triglyceride starting material and of the hydrolysis products. Greater than 95% of the radioactivity was found in the monoglyceride region of the TLC plates. The fatty acid analyses are shown in **Table 2.**

In vitro hydrolysis rates

Initial screening of six fats of various chain length and triglyceride structure was performed with enzymes provided by a combination of bile and pancreatic fluid obtained from a rat with a common duct cannula (8). The digestion medium contained per liter 3.9 g $CaCl_2 \cdot 2H_2O$, 58.9 g NaCl, and 0.3 g histidine HCl and was adjusted to pH 9.0. Fifty-five mL of this medium, 1 mL of substrate oil, and 0.1 mL of bile-pancreatic fluid were added to a 500 mL roundbottom flask. The contents were stirred with a motor-driven propeller stirrer at a rate that maximized the rate of hydrolysis in this system. The electrode-measured pH was maintained at 8.9–9.0 with the addition of 0.1 N KOH. A drop of substrate was added each minute to ensure that the reaction was not substrate limited. Triacetin was initially added at a volume of 3.5 mL and the additions were 1 mL.

The comparison of the rates of hydrolysis of 8L8, MCT, sunflower oil, and the Captex series of fats was made with a commercially available porcine pancreatic powder (steapsin; ICN Pharmaceuticals, Plainview, NY). The digestion medium was the same as that described above with the exception that the concentration of $CaCl_2 \cdot 2H_2O$ was 3.2 g/L. Seventy mL of this medium, 2 mg of oleic acid, and the substrate (0.25–4.0 mL) were added to a roundbottom flask (three port) and then emulsified by vigorous shaking with a wrist-action shaker for 10 min. The flask was then fitted with pH electrode, titrant delivery tube, and propeller stirrer. The reaction was initiated by delivery of 7.5 mg of enzyme (in 0.5 mL of digestion medium) into the stirred emulsion. The pH was maintained at 9.0 by the addition of 0.1 N KOH delivered with a Metrohm pH stat and titrator system (Brinkmann Instruments, Westbury, NY). The linear portion of the plot of added base vs time during the first 1–4 min of the reaction was used to determine the rate of fatty acid produced per minute for each fat.

TABLE 1
The fatty acid composition (weight %) of the Captex 810 series

Captex series	Linoleic	Octanoic and decanoic	Other
810A	10	80	10
810B	25	60	15
810C	35	46	19
810D	45	32	23

TABLE 2
Analysis of fatty acids in 8L8, 8L•8, and OL•O and in the products resulting from lipase digestion (fatty acids from the 1 and 3 positions and 2-monoglycerides)*

	Observed (theory), mole %		
	Triglyceride		
Compound	Octanoic	Oleic	Linoleic
8L8	65 (67)	0 (0)	35 (33)
8L•8	65 (67)	0 (0)	35 (33)
OL•O	0 (0)	67 (67)†	33 (33)
	2-Monoglycerides		
8L8	0 (0)		100 (100)
8L•8	0 (0)		100 (100)
OL•O	0 (0)		97 (100)
	Fatty acid		
8L8	100 (100)		0 (0)
8L•8	97 (100)		1 (0)
OL•O		100 (100)†	0 (0)

* 8L8 and 8L•8 are 2-linoleoyl-1,3-dioctanoyl glycerol and 2-[1-^{14}C]linoleoyl-1,3-dioctanoyl glycerol, respectively. OL•O is 2-[1-^{14}C]linoleoyl-1,3-dioleoyl glycerol. Observed values are given in mole percent followed by theoretical values in parentheses.

† Included 5% palmitic and 3% stearic acid in oleic starting material.

The comparison of the rates of hydrolysis of 8L8, MCT, sunflower oil, and the Captex series of fats was made with TAG as the substrate. Triacetin was initially added at a volume of 3.5 mL and the additions were 1 mL.

Statistical analysis of hydrolysis data

The linearity of the curves from the initial screening hydrolysis (**Fig 1**) was established by the determination that both intercepts and quadratic coefficients were not significantly different from zero in a multiple regression analysis for each curve. The slopes for each curve were then determined by linear regression through the origin. Each of these regressions had an R^2 value > 0.99. The slopes were statistically compared by multiple linear regression through the origin allowing for a different slope for each fat.

The hydrolysis rates of 8L8, MCT, sunflower oil, and the Captex series (**Figs 2 and 3**) were analyzed by one-way analysis of variance (ANOVA) comparing the maximum

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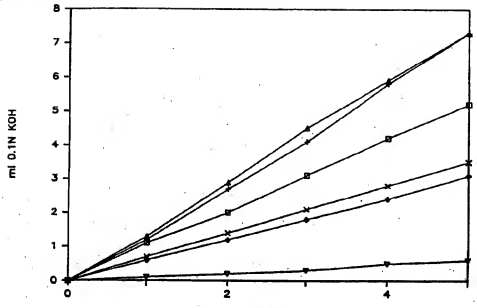

FIG 1. Fatty acid production (in terms of KOH added to maintain pH) of triglycerides of various chain length and structure hydrolyzed by rat pancreatic enzymes. Triglycerides are designated by the following symbols: Δ, 2-oleoyl-1,3-dioctanoyl glycerol; +, triheptanoin; □, medium-chain triglyceride (MCT); ×, tridecanoin; ◇, 2-oleoyl-1,3-diacetyl glycerol; and ▽, triacetin.

rates for each fat. Two methods for the determination of the maximum rates were found to yield the same results in the ANOVA. These methods were *1*) the values of the rates at the two highest levels of triglyceride substrate for each fat and *2*) the asymptotic value A estimated from the model, rate = $A[1 - e^{-kt}]$, where t is the triglyceride volume and k is the estimated shape parameter, This model was found to fit the data well with R^2 values > 0.95 for each of the seven fats.

Animal studies

We followed a procedure similar to that used by Greenberger (9) in comparing the absorption of medium- and long-chain triglycerides. The animals used in our trials were young adult, male Sprague-Dawley rats (Charles River Laboratories, Portage, MI). HEW Guidelines (NIH 85-23) for the care and use of animals were followed. Animals were anesthetized with Sodium Nembutal. After laparotomy the small intestine was ligated ~20 mm distal to the common duct. The small intestine was then irrigated below the ligation with 50 mL of 0.85% saline introduced by syringe to reduce residual enzyme activity. The small intestine then was ligated a second time 20 mm proximal to the cecum. Two mL of an emulsion containing 2-[1-^{14}C]linoleoyl-1,3-dioctanoyl glycerol (8L+8) or 2-[1-^{14}C]linoleoyl-1,3-dioleoyl glycerol (OL+O) with total radioactivity of ~100 000 cpm was then injected by syringe just below the proximal ligature. This emulsion was prepared with 0.2 g triglyceride, 0.02 g egg lecithin, 0.3 mL of 1% bovine serum albumin, and 1.48 mL Krebs Ringer solution (pH 7.4), which was sonicated for 30 s with a Heat System-Ultrasonics Inc processor (Plainview, NY). The emulsion dose delivered ~30 mg of triglyceride to each animal.

After 45 min the animal was killed and the isolated loop was removed. The contents of the loop were recovered by gentle expression of the intestinal segment and rinsing with 20 mL of 0.85% saline into a flask containing 50 mL hexane and 5 mL concentrated hydrochloric acid. The rinse was extracted twice with pet ether:ethyl ether (50:50), water washed, dried with sodium sulfate, and concentrated for analysis. Aliquots of the contents were taken for scintillation counting to determine the fraction of the dose remaining in the small intestine. The extent of hydrolysis of the intestinal fat was determined by the distribution of ^{14}C on a TLC plate after elution with petroleum ether:ethyl ether:acetic acid (75:25:1). The region corresponding to triglyceride was identified with standards and scraped into scintillation vials, and radioactivity was quantitated as the fraction of total activity on the plate.

Results

In vitro lipase digestions

A comparison of the rates of hydrolysis of triglycerides representative of short-chain,

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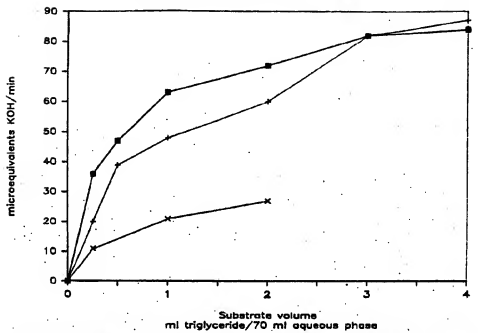

FIG 2. The rates of steapsin-catalyzed hydrolysis of triglycerides as a function of mass. □, medium-chain triglyceride; +, 2-linoleoyl-1,3-dioctanoyl glycerol; ×, sunflower oil.

medium-chain, and long-chain fatty acids by rat pancreatic enzymes is shown in Figure 1. The rates of the medium-chain fatty acids (MCT, triheptanoin) were higher than the long-chain (soybean oil) and very-short-chain (triacetin) fatty acids. Moreover, the rate remained high when the medium-chain fatty acids occupied the 1 and 3 positions of the triglyceride and the 2 position was esterified with a long-chain, oleic acid. The rate of hydrolysis of each fat differed from that of each of the other fats in this analysis ($p < 0.05$).

We also compared the rates of hydrolysis by steapsin of 8L8, MCT, sunflower oil, and a series of triglycerides containing medium- and long-chain fatty acids that were random in structure (Captex series). In each case the mass of the fat was increased to provide the maximum interfacial areas and the resultant maximum rate that could be obtained with the invariant conditions of mixing, emulsion preparation, and enzyme concentration. The graphs of rate vs substrate mass are shown in Figures 2 and 3. These results again demonstrated the rapid hydrolysis of the medium-chain fatty acids. The rate of hydrolysis of the Captex series increased with increasing amount of medium-chain fatty acid. A comparison of the maximum rates of hydrolysis of the fats in Figures 2 and 3 showed that MCT and 8L8 were not different and Captex 810C and 810D were also not different ($p > 0.05$). All other comparisons yielded statistically significant differences ($p < 0.05$).

Isolated loop studies

The studies of 8L•8 and OL•O injected into irrigated, isolated intestinal loops showed 8L•8 to be significantly more hydrolyzed and absorbed than OL•O. Measurements were obtained from the ^{14}C remaining in the intestinal contents 45 min after injection.

Absorption calculated as percent of ^{14}C dose was 44.9 ± 2.8 for 8L•8 and 16.8 ± 4.7 for OL•O (mean ± SEM, $n = 10$, $p < 0.05$). The percent unhydrolyzed triglyceride was calculated from thin-layer chromatographic anal-

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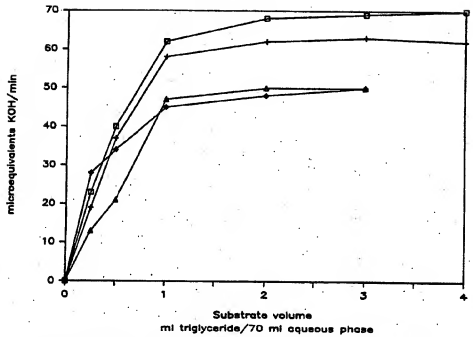

FIG 3. The rates of steapsin-catalyzed hydrolysis of triglycerides of random structure and various long- and medium-chain fatty acids composition (Captex 810 series). Weight percent linoleic acid and combined octanoic and decanoic acids, respectively: □, 10, 80; +, 25, 60; ○, 35, 46; and △, 45, 32.

ysis of the lipid radioactivity to be 11.1 ± 3.7 for 8L•8 and 48.3 ± 2.5 for OL•O (mean ± SEM, $n = 10$, $p < 0.05$).

Discussion

Randomly esterified triglycerides of medium- and long-chain fatty acids have shown promise both in parenteral (10–12) and oral nutrition (13). The data we have presented help to describe events in the intestinal lumen that apply to the oral use of these triglycerides.

The rate of hydrolysis of triglycerides with medium-chain fatty acids as the lipase-hydrolyzed esters in the 1 and 3 positions by rat pancreatic lipase is faster than that of typical long-chain fatty acid triglycerides (Figs 1 and 2). The 1,3-dioctanoyl fats may be synthesized to provide the most desirable features of medium- and long-chain fatty acids for use as nutrients in cases of pancreatic insufficiency. Ease of hydrolysis and absorption of the medium-chain fatty acids can be combined with delivery of EFA as the well-absorbed 2-monoglyceride.

The Captex series of random triglycerides hydrolyzed more rapidly as the fraction of their fatty acid content made up by octanoic and decanoic acids increased (Fig 3). The maximum rate of hydrolysis of these random structures with fatty acid compositions comparable to that of 8L8 (Captex 810C and 810D, 0.5 μEq/min) was ~60% of that of the 8L8 (0.88 μE/min; Figs 2 and 3). A high level of digestion and absorption of Captex 810B and 810D was demonstrated in cystic fibrosis patients with pancreatic insufficiency (13). These random triglycerides presumably include 10–15 mol% as 8L8. From its higher rate of hydrolysis, 8L8 may be the optimum form for linoleic acid absorption in pancreatic insufficiency.

The absorption of the products of hydrolysis of 8L8 presumably follows the normal routes of absorption—octanoic acid via portal vein

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and 2-linoleoyl glycerol via the lymphatic route. In this manner such a fat could provide > 50% of fat calories as long-chain fatty acid that will be absorbed efficiently even in cases of pancreatic insufficiency. Any portion of the long-chain fatty acids could comprise essential fatty acids.

References

1. Holt PR. Studies of medium chain triglycerides in patients with differing mechanisms for fat malabsorption. In: Senior JR, ed. Medium chain triglycerides. Philadelphia, PA: University of Pennsylvania Press, 1968:97–107.
2. Bach AC, Babayan VK. Medium chain triglycerides: an update. Am J Clin Nutr 1982;36:950–62.
3. Hirono H, Suzuki H, Igarashi Y, Konno T. Essential fatty acid deficiency induced by total parenteral nutrition and by medium chain triglyceride feeding. Am J Clin Nutr 1977;30:1670–6.
4. Mattson FH, Volpenhein RA. The digestion and absorption of triglycerides. J Biol Chem 1964;239:2772–7.
5. Mattson FH, Volpenhein RA. Synthesis and properties of glycerides. J Lipid Res 1962;3:281–96.
6. Mattson FH, Volpenhein RA. The use of pancreatic lipase for determining the distribution of fatty acids in partial and complete glycerides. J Lipid Res 1961;2: 58–62.
7. Metcalfe LD, Schmitz AA, Pelka JR. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal Chem 1966;38:514–5.
8. Mattson FH, Volpenhein RA. Hydrolysis of fully esterified alcohols containing from one to eight hydroxyl groups by the lipolytic enzymes of rat pancreatic juice. J Lipid Res 1972;13:325–8.
9. Greenberger NJ, Rodgers JB, Isselbacher KJ. Absorption of medium and long chain triglycerides: factors influencing their hydrolysis and transport. J Clin Invest 1966;45:217–27.
10. Babayan VK. Medium chain length fatty acid esters and their medical and nutritional application. J Am Oil Chem Soc 1981;58:49A–51A.
11. Maiz A, Yamazaki K, Sobrado J, et al. Protein metabolism during total parenteral nutrition (TPN) in injured rats using medium chain triglycerides. Metabolism 1984;33:901–9.
12. Heird WC, Grundy SM, Hubbard VS. Structured lipids and their use in clinical nutrition. Am J Clin Nutr 1986;43:320–4.
13. McKenna MC, Hubbard VS, Birri JG. Linoleic acid absorption from lipid supplements in patients with cystic fibrosis with pancreatic insufficiency and in control subjects. J Pediatr Gastroenterol Nutr 1985;4: 45–51.

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